Transcript for the Nested Dichotmous Models Module of the on-line BMD Models Training

Slide 1. Welcome to the online benchmark dose modeling training session for the nested model.

Slide 2. As we mentioned previously, the online benchmark dose training consists of 5 training modules.
These five training modules are the introduction to dose response modeling, dichotomous models, a cancer model, continuous models, and nested models.
In this session of the online BMD training, we are going to discuss the benchmark dose modeling for nested dichotomous data.

Because many of the basic concepts used in this session have been covered under the introduction to BMD and the dichotomous model training sessions, it is highly recommended to view those sessions before you proceed to this session.

Slide 3. What are nested data? In the nested data, the observation unit is different from the exposure unit.
In a regular experiment design, the exposure unit or subject is the same unit as those used in the observation. For example, in a regular study, if we have 50 animals per dose group, we usually observe a particular endpoint in each of the 50 exposed animals. If they are the quantal or dichotomous response, you would have a yes or no response for each animal that has been treated in the experiment. So, in each dose group, the observation would be a certain number of responding animals out of the 50 animals treated.

Slide 4. Let's look at the nested data setting. In a nested study, a group of animals are treated with a particular dose of chemical, and the end point examination is conducted in a sub set of subjects from each treated individual. Thus, the observation sample size is larger than that of the original treated animals. A good example of nested data is the neonatal response data obtained from developmental studies. When each individual mother is treated with a chemical, the response is usually observed in pups of the treated mother. Because each mother may produce 15-20 pups, if you have 20 treated mothers, you would have 20 observations from each mother. Thus, your final observation sample size is much larger than the number of treated mothers.

Because the pups are exposed through the mother, there might be a commonality among the pups in each litter. If one mother is exposed to less of the chemical than the other mothers due to individual variation in the toxicokinetics, then all the pups from that mother are exposed to less dose as well. So, there will be a correlation in the observed responses among the pups within each litter. Because the sample from the pups is larger than the number of mothers which directly received treatment, the nested data will provide an enhanced statistical power for data analysis.

Nested data can contain dichotomous response or continuous response data. The current EPA's BMDS software only contains nested models for modeling dichotomous data, therefore, we are going to focus our discussion on nested dichotomous data only.

Slide 5. Here we provide some examples of nested response data that you will see from developmental studies.

Developmental effects could range from skeletal structure change, delayed ossification in the bone, or organ structural change **to** malformation. Since all those observations are made in pups, but not in the mothers, these data should be considered nested data. Therefore, the data analysis and dose response modeling should use nested data approach.

The response data from the exposed mothers should be considered regular data instead of nested data, and their analysis should be the same as we will do with the dichotomous model as we discussed in the dichotomous training module.

Slide 6. The dose response curve for nested dichotomous data will be similar to the regular dichotomous data.
Here we are focusing on dichotomous data. In this case, the Y axis represents a percentage of animals response, and x axis represents the treatment dose. The unit for dichotomous data on the y axis is the percent of animals responded which range from 0% to 100%. There is always an upper limit for the dichotomous response. In contrast, the response unit for a continuous response will have a specific measurement unit that depends on the endpoint, and it usually does not necessarily have an upper limit.

Slide 7. To conduct benchmark dose modeling for the nested model, we will use the same procedure we used for the regular dichotomous data.

First, we need to determine whether the data we have are worth modeling. Here, the same evaluation criteria we used for the NOAEL approach also apply.
For example, in order to do a good dose-response analysis, you need a quality study.

In addition, you need to pay attention to the experimental conditions they used to generate these data. Ideally, the experiment should be conducted for an appropriate duration and through an appropriate route of exposure.

You also need to pay attention to the organ or tissue where the response was observed. That means the measured endpoints of concern. If you know the target organ for a particular chemical toxicity, but a study did not evaluate the effects at that organ or tissue, this study might be of limited value because it did not provide dose response data at the target organ, even though it did measure the effects at other organs or tissues.

Once you make your judgment about the dataset, ideally you are supposed to model all the endpoints or adverse effects possible. Once you have modeled all endpoints, then you can determine which response is most sensitive based on the dose-response information.

Slide 8. Secondly, for reliable dose response modeling, the data should contain a significant dose-related trend, and they should have at least three data points.
In addition, you also want that data set to contain the data points that had a response close to the benchmark response (BMR) you are going to use. That will also provide a reliable estimate of the benchmark dose or BMD, OR the lower limit of benchmark dose or BMDL. If you have a data point that is far away from the BMR, the estimated BMD will rely heavily on extrapolation based on the curve fitting. This extrapolation may produce some uncertainty in the estimated BMD.

Slide 9. If you have multiple endpoints, you should model all the responses with statistical significance, if feasible.

When you have limited resources available, you will need to at least model the important sensitive endpoints. To do so, you will have to identify the potential most sensitive effects based on the lowest observed adverse effect level or LOAEL. We recommend modeling all endpoints with a LOAEL within a 10-fold range above the lowest LOAEL you have identified. This way, you limit the possibility of missing the sensitive response at low dose if the Dose-Response curve is very steep.

Once you have modeled the data, if the curve fitting made a sacrifice at the low dose range in order to accommodate the overall data points, you may consider dropping the high dose data points in order to improve the model fitting to the low dose range. As we discussed in other sessions, benchmark dose modeling uses a mathematic model to predict dose response, especially at the low dose range where the benchmark response is.

Slide 10. Here are the common 6 steps of the BMDS analysis procedure.
Step 1 is to chose a Benchmark Response, this is also called the BMR.

Slide 11. Measurement of increased risk for nested dichotomous data is similar to the regular dichotomous data we mentioned in the dichotomous model training session.

One way to measure the increased risk is additional risk. The additional risk is the increase in risk from the control dose (0) to treated dose (d), and it is calculated as P(d) - P(0). That means the probability of response at dose (d) minus the probability of response at the control dose (0).

Another measure of increased risk is extra risk. The extra risk is expressed as the difference in terms of response of the treated dose minus the response of the control dose, divided by 1 minus the control response. Or we can say this is the percent of response you can really achieve. For example, if there are 100 animals in each dose group and the background response in the control group is 30%, then 30% of the animals will exhibit the response even without chemical treatment. Thus, at least 30 animals will show the response whether treated or not, and only 70 animals can really change their response when they receive the treatment. The extra risk is the percent of the 70 animals affected after the treatment.

EPA recommends to use the extra risk in BMD modeling.

Slide 12. Although, an extra risk of 10% is usually used as a default BMR for regular dichotomous data, an extra risk of 5% is used to approximate the NOAEL for many developmental studies. A developmental study will provide extra statistical power because the sample size has increased by use of pups as the observation subject. Therefore, a 5% response increase in risk in those pups actually corresponds to a NOAEL from a regular developmental study as shown by Allen et al. 1994. This is why for nested data from a developmental study, we may chose 5% instead of 10% as our BMR. EPA recommends that when dealing with quantal data, no matter what BMR you are using, 10% extra risk should always be used as a comparison.

Slide 13. Step 2, once we define the BMR, we then need to select the model, set parameters, and run the model.

Slide 14. The EPA BMDS software provides three nested dichotomous models. They are Nested Logistic Model, the NCTR Model, and the Rai & Van Ryzin Model.

Slide 15. The goal of BMD modeling is to find the model that fits the data the best. Keep in mind that nonlinear models do not necessarily have a biological interpretation.

The criteria for final model selection will be based solely on whether various models describe the data.

Slide 16. Once you have chosen a model, you must select the parameters. Each model has its own parameters to choose from. However, for nested data modeling, there are some specific model parameters we need to consider in addition to the parameters we encountered in regular dichotomous BMD models.

Slide 17. Because pups from the same litter were exposed to the chemical in the same mother, these pups are usually similar in some way. Thus, it is common for the responses of the pups in a litter to be more similar to each other than to pups of another litter. This is called intralitter correlation, or litter effect, and this is a major issue in the dose response analysis of nested data. The nested models in BMDS software incorporate two parameters to account for this intralitter correlation; they are litter specific covariate and intra-litter correlation.

Let’s show you in a diagram.

Slide 18. This diagram shows how the intralitter correlation is controlled. In each toxicity study, a treatment causes a response. In contrast to random variance among individuals in the dose group, the individual variance in nested pups consists of two factors: random variance and intralitter correlation. The intralitter correlation can be further attributed to two conditions: Pre-treatment conditions, and treatment conditions.

Slide 19. The parameter used in the BMDS software to control for the pre-treatment conditions is called the litter specific covariate. The parameter to control the treatment conditions is called the intra-litter correlation.

Slide 20. First, let's discuss the litter-specific covariate. This parameter controls for pre-treatment conditions within each litter.

Slide 21. The litter specific covariance takes into account the condition of the dam before the treatment. This mother specific condition might explain some of the observed effects in each litter. For example, if the mother had ten pups in a litter, she can provide more nutrients for each pup, than a mother who has 20 pups. However, this mother condition should not be affected by treatment, otherwise, a treatment –related response might also get controlled. Anything affected by treatment will be dealt with by another parameter.

The commonly used data for the Litter Specific Covariate are the litter size, dam weight, and number of implantation sites. The litter specific covariate is only used to control the factors before treatment. Therefore, we need to carefully evaluate if the litter size we are going to use has been affected by the treatment.

Slide 22. Once you select the model type you will receive this screen to select parameters. Check "use litter specific covariate" to have this parameter applied to the model. Check "do not use litter specific covariate," if you do not want this parameter applied to the model.

Slide 23. How do you determine whether particular parameters such as litter size are affected by the treatment? First we need to understand the physiological process of fetal development.

This is diagram shows the time-line of a regular developmental study.
Following a successful mating, the fertilized eggs migrate into the uterus and get implanted around gestation day 5 or GD5. Then they start to develop into fetuses. In most common developmental studies, treatment starts from gestation day 6 and ends on gestation day 15, because this is the period for organ formation, and it allows us to easily observe the treatment effects by visual inspection. We can often see shortened limbs or structural changes in the internal organs or skeleton by visual inspection.

Exposure to developmental toxicants before GD6 will often prevent a successful implantation, therefore, there are no forms of fetuses at the end of study. These forms of fetuses could include early resorption tissue residues, and dead or live pups at the term. During GD5 to a little after GD6, toxicity to the pups could result in an early resorption.

If the treatment starts after GD6, the fetuses may survive, but the organs and body structure can be affected. In addition, some fetuses might not survive the treatment which results in prenatal death. In this case, you will see mature size dead pups.

The mothers are usually sacrificed on GD 20. That is one day before the regular parturition date, so that we can make observation on all of the matured pups. At this time, the uterus is opened surgically, and total implantation numbers can be determined by

summing the number of early resorption sites, and live and dead pups. The litter size usually refers to total number of live pups at the end of the experiment.

If the treatment starts after GD6, implantation is the best way to measure the litter size because the total number of resorption, and live or dead pups will not be affected by the treatment. However, these data are not always available from a developmental study. On the other hand, if the treatment occurred before mating, total implantation could be affected by the treatment, therefore, it would not be an appropriate parameter to use as the litter specific covariate.

Commonly, a developmental study will only include litter size, which only includes live pups. Since this parameter could be influenced by toxicity effect due to treatment at the early gestation, these data can only be used when there is no evidence of such effect.

Slide 24. As a summary, *Litter size* is an appropriate litter covariate, as long as there are no dose-related prenatal deaths or resorptions, because it is a measure that is generally proximate to the endpoint being measured. The number of live fetuses in the litter at term would not be an appropriate measure if there are dose-related prenatal deaths or resorptions.

The number of implantation sites is often a better alternative to litter size if dosing begins after implantation. However not all studies provide these data.

Slide 25. There are three considerations when deciding whether or not to use the Litter Specific Covariate. First, does the Litter Specific Covariate appear to be affected by the dose? This can be found in the study design.

Second are thetas non-zero? Thetas are the parameters used in the BMD software for the Litter Specific Covariate. We can pre-run the data with the setting for litter specific covariate and examine the resulting theta values estimated by the model. If thetas are not close to zero, then theta may be important. As in regular statistical analysis, the estimated thetas have best estimate means and standard errors. If a range of the best estimate of a theta plus or minus the standard error includes 0, that indicates a non significant theta.

Third, when the Litter Specific Covariate is excluded, does the model fit become worse? We can pre-run the model with the Litter Specific Covariate, then run it again without this parameter. The additional parameter of litter specific covariate should improve the model fitting to the data. This can be evaluated by comparison of the AIC values and the scaled residuals. If introducing litter specific covariate does not improve the model fitting, we don't need this parameter.

Thus, if we answered "no" to these questions, we do not need to use the litter specific covariate. If a clear "yes" is the answer for all of these questions, using the Litter Specific Covariate will improve the model fitting; therefore, we should use this parameter.

Slide 26. Let's look at an example.

Here we use the sample data file that comes with the BMDS software called nested.set.

Let's assume there is no evidence that litter size is affected by chemical treatment, so there is no problem based on biological consideration. Since there is no treatment related effect on litter size, we can use it as a Litter Specific Covariate for the pre-run.

Slide 27. This is the estimated parameter summary table that you will see after the pre-run. As we mentioned previously, the thetas are the parameters for the Litter Specific Covariate. The best estimate thetas are listed in green, along with their corresponding standard errors. We need to examine whether the estimate mean plus or minus the standard error covers zero. In this particular case, the ranges of thetas + or – the corresponding standard errors covers 0. So, the estimated thetas are not necessarily significantly different from zero, thus we should run the model without the litter specific covariate. Note that the AIC value in this case is 560.

Now, let's run the model again without the Litter Specific Covariate to see what happens when the thetas are removed.

Slide 28. This is the estimated parameter summary table you will see after the run without the Litter Specific Covariate. After removing the thetas, the AIC value is decreased from the previous 560.5 to 556.9. Remember, the smaller the AIC, the better. Thus, introducing the litter specific covariate did not improve the model fit. We do not need this parameter.

Slide 29. From the type model run screen, you will notice a choice of using Fixed Litter size. Previously, the BMDS user manual explained when to use the overall mean and when to use the control group mean.

Slide 30. However, current EPA practice is to use the overall mean for all nested model runs. This choice of parameter may be removed from future versions of BMDS software.

Slide 31. The other nested model parameter is intralitter correlation. As discussed previously, the intralitter correlation is used to control the effect after treatment starts.

Slide 32. This is the same nested model screen shot that we saw earlier for parameter selection. You can chose either "estimated intralitter correlation" or "assume Intralitter Correlations zero."

Slide 33. Intralitter Correlation describes the similarity among pups of the same litter, and it is described by phis in the BMD models. For this parameter, typically we will have to pre-run the model to decide whether to use this parameter just as we did with Litter Specific Covariate.

There are two considerations:

First, are the estimated phis close to 0 in the pre-run? Phis are the parameters that denote Intralitter Correlation. We need to examine whether the range of the best estimate of phis plus or minus the corresponding standard errors covers zero. The values will be not significant if the range covers zero.

Second, when Intralitter Correlation values are set to 0, is the model fit unchanged or improved? Additional parameters should improve the model fitting to the data. Otherwise, we should not use it. We can look at the AIC values and scaled residuals to compare for model improvement just as we did for Litter Specific Covariate.

If you answered "yes" to both questions, you do not need to use the Intralitter Correlation.

Slide 34. This is the estimated parameter summary table we will see after the pre-run of the sample dataset using the Intralitter Correlation. As mentioned previously, if the Phis are 0, introducing phis will not improve the model fitting. Note the AIC value of 566 and P value of 0.9934. Run the model again using the "assume Intralitter correlations zero'" to see what happens when phis are removed.

Slide 35. This is the estimated parameter summary table you will see after running the model without using the Intralitter Correlation. Note that the AIC and P values do not change at all. Since there is no influence on the overall data fitting, we do not need to "estimate intralitter correlations."

Slide 36. In summary, we need to decide whether to use the Litter Specific Covariate based on information from the experiment, and to determine whether there is any treatment related effect on the parameter that will be used as the litter specific covariate, such as litter size. If there is no information suggesting treatment related effect, run all the nested models with and without Litter Specific Covariate. Examine theta values and model fits to determine whether we should use this parameter.

For intralitter correlation, run all of the nested models with or without this parameter, and examine the phi values and model fits to determine whether we should use the Intralitter Correlation in the final model.

Then rerun the final model with the appropriate parameters, and summarize results to include the AICs and scaled residuals.

Slide 37. Once we have chosen a model, and made the parameter selection, we will run the final model.

We have to deal with each situation all over again for each nested model. However, there are similarities among these models, so you will only need to run each model once because those decisions are very easy to make in the next 2 model runs. Then, we run the model and look at the model fit to the data.

Slide 38. Once we run the model, we will look at the model fitting results. We will visually examine the model fitting, evaluate the goodness of fit p value, local scaled residuals, and AIC.

Slide 39. First we will examine the model fitting to the data points.

Slide 40. This is the dose response curve generated from our sample data set. We look at curve fitting for the 4 data points, paying special attention to the low dose, which is 25 mg/kg dose group. We find that the model fits all the data points very well, and the curve also fits the low dose range which is close to the BMR.

Slide 41. Now, let's evaluate the statistics provided by the BMDS software. We will evaluate the overall model fit by using goodness of fit p value. The acceptable goodness of fit p value for BMD data modeling is greater than 0.1.

Slide 42. The goodness of fit p value is provided in the lower summary table of the output file, right above the BMD estimate. For this particular data set, the P value is 0.9344, indicating a very good model fitting to the data set.

Slide 43. As we discussed in other BMD training sessions, we can also use the scaled residuals to evaluate the model fit, especially at the response close to the BMR. The absolute value of the scaled residuals should be less than 2 for a good model fit.

Slide 44. In the nested model, the scaled residual is obtained a little differently from the other BMD dichotomous models.
In the regular dichotomous models, each data point has its own scaled residual. In contrast, for the nested dose BMD modeling, there are numerous scaled residual values for the same data point. So, the selection of the scaled residuals is determined not only by the data point that should be close to the BMR, but also by the estimated litter specific covariate.

First, we need to identify the data point closest to the BMR. For the same data point, there will be multiple observation. We need to identify the observations with the estimated litter specific covariate closest to the mean litter specific covariate of all the data. The mean litter specific covariate can be found in the BMD output sheet as we will show in an example. When there are multiple scaled residuals with the same litter specific covariate, an average of these scaled residuals will be used.

Slide 45. **Here is an example:**

This is a part of the scaled residual summary table from a nested model run with the sample data. At the lower end of the table, there is a statement that says "to calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all of the data," which is 11.65 for this data set.

There are 4 dose groups in this dataset, we need to identify the data point closest to the BMR. The 25 mg/kg dose group is the closest to the BMR as shown in the curve fitting. As shown in this slide, each litter has their own litter specific covariate, we need to identify the litter with the litter specific covariate closest to the 11.65 mean value. We find that one litter has a residual of 12, which is closest to the mean value among all the litters in that dose group. So we will use the scaled residual value from this particular litter. In this case, there is only one litter that has the litter specific covariate closest to the mean value. Sometimes, we might see several litters with the same litter specific covariate. For example, if we had 3 litters with the same litter specific covariate of 12, then you would take an average of the scaled residuals from these three litters as the representative scaled residual for this dose group.

Slide 46. Here is a summary of the nested model runs for the sample data. Among the three nested models, the Nlogisitic model provides the highest p value and the lowest AIC, while the NCTR model and Rai & Van Ryzin model provide a relatively smaller scaled residual than the NLogistic model at the 20 mg/kg dose group. Please note that we need to compare the absolute values for the scaled residuals. Therefore, it is recommended to use an average of BMD or BMDL from these three models as the final result for this data set.

Slide 47. Let's do a step-by-step nested model run. You can practice this on your computer while you are viewing this presentation. You can stop anytime and compare your results with our presentation.

Slide 48. This is the study information for the sample data we are going to use. Every time we run a nested model we need to pay attention to experiment design and when the treatment was given to the animals so that we can determine whether to use data such as litter size or number of implantation sites as the litter specific covariate.

This is an inhalation study conducted in mice. The treatment started on gestation day 6 and ended on gestation day 15. So, the treatment was from GD 6 to GD 15. Half of each litter was prepared for the skeletal examination. Exposure to the chemical caused an increased anomaly in the cervical rib.

The sample data file is provided on this web site, as the file name of nested1.set.

Slide 49. This is the summary table published in the original paper with the critical endpoint highlighted. The treatment groups consisted of 3 control groups, 1000, 2000, 5000, and 15000 ppm concentrations. The endpoint of the cervical rib response was expressed as dichotomous data.

Slide 50. There are two ways to transfer the data from a spreadsheet to BMDS. The first and also the easiest way is using the copy and paste as we mentioned in continuous model training session. Another way is to electronically import the spreadsheet data into the BMDS, and this is a better method for transferring a large amount of data from the spreadsheet.

Because the nested model input contains a lot of data, entering the data into BMDS software manually can be difficult. Therefore, it is easier to prepare the original data in an excel spreadsheet and then transfer them into BMDS. In this example, we are going to use the electronic data transferring method.

To prepare the spreadsheet for the BMDS data import, only one worksheet should be created in each excel file. Then, the saved spreadsheet can be directly transferred into the BMDS.

Slide 51. Here is how we prepare the data in the excel spreadsheet.

In this data sheet, we entered titles in the first row, then we input data for the individual pups in each row.
For example, in the first data row, we entered 0 for the control dose, 6 for the litter size, 0 for the response in the first litter, and 6 in the covariate column. Each row indicates responses observed from a litter, and response 0 means no pups in this litter responded. Covariate column is basically the litter size data that we are going to use as litter specific covariate. We need to repeat the litter size data in a separate column so that the BMDS can recognize it as an independent value. Please note that there is only one worksheet in this spreadsheet file as indicated in the slide. The BMDS will ask you whether you want to use information in the first row as the titles in the BMDS while you import the data. You need to respond according to your data entry in the sheet.

Slide 52. Once we have prepared the spreadsheet and saved the file, we can start the BMDS software, and use the open file function to open the Excel file you just generated. Then we can click the load/import button to import the data.

Slide 53. After we click the load/import button, the BMDS will ask which version of the excel spreadsheet was saved. Different versions of excel may have different definitions in the file structure. Select the appropriate version from the pull down menu. Another pop-up will ask: “do you want to use the first row as titles?” If you click yes, it will copy the first row information into the titles in the BMDS.

Slide 54. This is the dataset screen after the data are imported into the BMDS. The data on this screen should be identical to the original data in the spreadsheet file. Once we have successfully imported the data, we need to select the type of model we are going to use. Because we want to use the nested model, we will select nested from the pull down menu. Then in the model choice, we have three models available. Here we’ll use the Nested Logistic model as an example. Once we have made these choices, we then need to tell the computer how to assign data columns to each required data entry. In this case, we assigned NDOSE column to dose, LITSIZE column to litter size, response column to incidence, and covariate column to litter specific covariate. Once we assign those columns, we can now proceed to next step.

Slide 55. So far, we have started the BMDS, imported the data and assigned data. Now we are going to talk about the common 6 steps in BMD modeling. First we need to define the benchmark response that we are going to use.

Slide 56. As we mentioned previously, EPA recommends using Extra Risk. So we will use extra risk to measure the BMR.

The default BMR for regular dichotomous data is 10% extra risk. However, in the nested data we usually have more statistical power, so we can use something less than 10%. As we mentioned in the beginning of this presentation, 5% extra risk is usually equivalent to the NOAEL in developmental studies. So, here we are going to use 5% extra risk as the BMR.

Slide 57. Step 2. select a model, set the parameters and run the model.

We have selected the NLogistic model in the dataset screen. Now let's discuss the parameter settings.

Slide 58. For setting the litter specific covariate, we need to assess the effect of the concentration on the litter size. Litter size is the only data that can be used from the sample data set as the litter specific covariate. As we know, the litter size can be used as the litter specific covariate, as long as the treatment did not affect the litter size in the experiment.

Here let's look at the information on the litter size. Based on background information, we know that the treatment started on GD6. As we mentioned previously, the live litter size might be affected if significant resorptions or prenatal deaths occurred, causing a decrease in the litter size.

This table summarizes the concentration effect on the litter size. There were significant treatment related decreases in the number of live pups per litter in the highest concentration group. Because there were significant treatment related effects in the live pups per litter, especially in the high concentration group, it is not appropriate to use the litter size as the litter specific covariate in the nested BMD modeling for this data set. If we have the number of implantation sites, we may be able to use it. However, in this example, we don't have such data.

Slide 59. This is our previous dataset screen. As you can see in this dataset, we have the litter size information; however, based on our analysis, it was affected by the treatment. Therefore, even though this information is available, it is not appropriate for use as the litter specific covariate in this model run.

Slide 60. Now, let's run the model. In this model run, we will not use litter specific covariate, and will estimate "intralitter correlation." Remember for the intralitter correlation, we usually prerun the model, and based on the model results decide whether

to use the intralitter correlation in the final model run. So here we use the default estimate for the intralitter correlation.

Slide 61. Here is a summary table to fill out, and we already entered the BMD and BMDL. You should be able to find these values on your output sheet. Now, you need to look for the scaled residual values, AIC, and P Value from the BMDS output sheet.

Slide 62. Let's find the scaled residuals.

Do you remember that we need to find the mean litter specific covariate from the output sheet?

In this example, we find that the mean litter specific covariate is 5.27 for the data set.

Slide 63. Which concentration group had the response closest to the BMR? From this concentration response curve fitting, we can see that other than the control

Slide 64. So, we need to find the litters in the 1000 ppm concentration group that have the litter specific covariate closest to the mean litter specific covariate of 5.27. In this case, the closest estimated litter specific covariate in the 1000 ppm group is 5. Because there are three litters with the same litter specific covariate of 5, we averaged the scaled residuals from these three groups to gave a mean scaled residual of -0.7836.

Slide 65. Now, we put this number in the summary table. In addition, we also add the AIC value of 1065 and the goodness of fit P value of 0.1973.

Slide 66. This is the model fit with the original 5 data points. When we look at the curve fit, we can see a problem in the model fit at the high concentration. So, we should drop off the high concentration data point to see if we can improve the model fit.

Slide 67. Now, let's look at the concentration effect on the litter size again. Without the high concentration data point, the live pups per litter in the rest of the concentration groups are not significantly affected by the treatment. So we can try to model the data with the litter specific covariate setting.

Slide 68. This is the model fit after we removed the high concentration data point. Here we can see that dropping off the high concentration group significantly improved the overall model fitting to the data points.

Slide 69. Now, let's find the scaled residuals for this new data set. The mean litter specific covariate in this model run is 5.38.

Slide 70. Again, we need to look at the middle of the summary table from the output sheet to find the estimated litter specific covariate close to 5.38 for the litters in the 1000 ppm concentration group. We find that three litters have an estimated litter specific

covariate of 5, which is closest to the mean value of 5.38. The average scaled residual is -0.72.

Slide 71. Now we add the information from the second model run to the summary table. For this second run, there was an increase in the goodness of fit p value from 0.197 to 0.275, a significant increase in the AIC from 1065 to 1049, and a small decrease in the scaled residuals from 0.7836 to 0.7259. Therefore, the removal of the high concentration, significantly improved model fit. .

Slide 72. Here is a summary of the basic procedure for selecting the litter specific covariate and intralitter correlation,

First, decide on whether to use the litter specific covariate based on information from the experiment. If a biological consideration suggests not to use it, then do not use the litter specific covariate. Otherwise, use the model prerun to determine whether the introduction of the litter specific covariate improves the model fit. Use the same considerations to determine if the intralitter correlation improves the model fit. Rerun the model with the appropriate settings for the litter specific covariate and intralitter correlation, then summarize the results including the AIC, scaled residuals, goodness of fit p value, BMD and BMDL.

Slide 73. This is a summary table for the NLogistic model runs without the high concentration.

We used various model-fitting parameter selections. Removing the litter specific covariate from the default setting of using both litter specific covariate and intralitter correlation causes an increase in the AIC value from 1049 to 1053. This means the introduction of thetas improved model fitting to the data.

Also look at the effect of the parameter for intralitter correlation, phi. It seems that the removal of phis would further increase the AIC. Thus, the inclusion of phis also improves the model fit.

Remember, the smaller the AIC, the better.

In addition to the changes in the AIC values, removal of these two parameters also decreased goodness of fit p values.

In conclusion for the NLogistic model runs, the best model fit was obtained from the default settings of using both litter specific covariate and intralitter correlation.

Slide 74. So, the NLogistic model provided a good model fitting to the data. Next, we need to model the data with the other two models, NCTR and Rai & Van Ryzin models.

Slide 75. For other the two models, we applied the same procedure as the NLogistic model. Here is the summary of the model run results from all three nested models.

Similar to the Nlogistic model, the NCTR model and Rai & Van Ryzin model also provided a satisfactory model fit. Their goodness of P value, AIC and scaled residuals are all comparable to those from NLogistic model.

Once all the model have been run, we need to consider how to select the BMD or BMDL from these models.

Slide 76. First, we need to evaluate whether the BMDLs from these models are within a 3-fold range.

Slide 77. Let's look at the summary table again.

The BMDL values from these three models range from 217 ppm to 270 ppm, which are within a 3-fold range.

Slide 78. This suggests that we need to examine whether any one model fits the data better than the others.

Slide 79. From the summary table, we can see that all three nested models provide identical AIC values. Therefore, all three models fit the data equally well.

Slide 80. Therefore, we should consider combining the BMDLs from all three models.

Slide 81. Here is a summary of the nested models. All three models produced a similar model fit based on the comparison of the AIC, goodness of fit p values and scaled residuals. The average BMDLs obtained from these models should be used. The mean BMDL is 252, which comes from the average of 217, 270, and 270 ppm. So we can use this average value of 252 ppm as the point of departure, if the anomaly in the cervical rib is the critical effect.

Slide 82. This concludes of benchmark dose nested model training session.

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